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The Effect of Isoindoline on Survival of Rats Subjected to Acute Thermal Burn.

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THE EFFECT OF ISOINDOLINE ON SURVIVAL OF RATS SUBJECTED TO ACUTE THERMAL BURN

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THE investigation of burn shock has left many questions unanswered. The significance and the mode of action of neural and toxic factors in shock are still inadequately known. Of particular interest in this area is the finding of Simonart and his Belgian co-workers.²⁰ According to them, various proteins, upon being heated to above 80° C., acquire toxic properties for frogs and rabbits. Thus it appeared worthwhile to investigate the effect of various temperatures on burns of equal area. Simultaneously an attempt was made to supplement Laver's⁸ observation of the protective action of two ganglion blockers by the use of an additional long-acting blocking agent.

Isoindoline*—4,5,6,7-tetrachloro-2-(2-dimethylaminoethyl)—represents a departure from the symmetrical configuration around the methylene chain, as in the methonium series, and possesses a halogenated aromatic heterocyclic substituent,¹⁵ thus resulting in a longer-acting and more potent drug. When tested in the nictitating membrane preparation of the cat 0.05 mg. per kilogram produced the same 50 per cent ganglion blockade as 0.32 mg. per kilogram of hexamethonium; however, the intravenous LD₅₀ in rats was similar.¹⁵

Thus it seemed logical to test the efficacy of this agent in prolonging the life expectancy of rats subjected to a hot water burn. Moreover, an attempt was made to determine the influence of relatively small changes in the temperature of the burn—85° C., 90° C., 95° C.—of treated and control rats.

METHOD

The procedure utilized in the experiments was a slight modification of the method described by McCarthy.¹² Two hundred apparently healthy light-brown rats, of the Russian Mura strain, varying in weight from 150 to 250 grams, were used. It may be added that these rats were found to be particularly resistant. The animals were kept in wire cages at constant room temperatures (23° to 22° C.) and fed a diet of dried pellets and water, *ad libitum*.

Anesthesia was induced by placing the animal in a 3 L. bell jar through which a well-dispersed oxygen-ether mixture was passed for 4 minutes. In order to facilitate handling throughout the burning process, the rat was held around the neck by a piece of short rubber tubing attached to a pincette; the hindlegs and tail were grasped together in one hand. A scald was produced by immersing the back of the rat in a water bath at a constant temperature of 85° C.,

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*Ecolid is made by Ciba Ltd., Basle, Switzerland.

90° C., or 95° C. for exactly 35 seconds. Special precautions were taken to prevent burning of the neck or extremities. Excess water was removed after immersion by rolling the animals on an absorbent cotton towel. Sodium chloride, 0.2 c.c. of a 0.9 per cent solution (control), or 0.2 c.c. of a solution of 1 mg. in 1 c.c. of Isoindoline was injected into one of their lateral tail veins. The amount of Isoindoline was well in the intravenous LD₅₀ range for the rat (28 mg. per kilogram).¹⁵ The animals were selected at random for treatment or to serve as controls.

Following burning, the animals were replaced in a 3 L. glass jar with a 3 cm. aperture at the bottom to allow for the removal of urine and fecal material, and kept at room temperature. No food or water was given after the burn.

Survival was checked at half-hour intervals for the first 12 hours, then at 2-hour intervals. Rats that lived longer than 24 hours were sacrificed. All our animals were dissected within 2 hours after death. The burned skin was excised, cut in half, and a new incision perpendicular to the dissection was made in order to compensate for the curvature when determining the area of the burn. The perimeter of the burned area was traced into cellophane and determined by planimetry. The proportion of burned to unburned surface areas was expressed in percentages, according to the formula $S = KW 0.6$ ($K = 12.54$) as formulated by Lee⁹ for the white rat.

The mean survival in the two extreme weight groups (150 to 200 and 200 to 250 Gm.) were compared in all our three temperature classes and differences were found insignificant. In the 85° C. class t was 2.1; in the 90° C. class t equaled 1.95; and 0.55 was the t for our 95° C. group. The two extremes in burned surfaces, that is 23 to 26 per cent and 28 to 31 per cent, were also compared in our three temperature sets. The difference in mean survival time was found not to be significant, t being 0.3, 1.0, and 0.65 in the 85° C., 90° C. classes. These findings verified those of Laver.⁸

All our results include animals weighing 150 to 250 Gm. with a burn of 27 ± 4 per cent surface area.

RESULTS

Table I summarizes our findings. Analysis of these results supports a hypothesis that intravenous injection of 0.2 mg. of Isoindoline immediately post burn significantly prolongs the mean survival time in all three temperature ranges investigated. As can be seen, t was 11, 22, and 11 in the 85° C., 90° C., and 95° C. groups, respectively, p being less than 0.001 in all three categories.

TABLE I. SUMMARY OF FINDINGS

BURN TEMPERATURE	NUMBER	MEAN AREA BURNED (PER CENT)	MEAN WEIGHT (GRAMS)	MEAN SURVIVAL TIME (MINUTES)	t	p
85° C. Treated	27	27.1 ± 1.3	215 ± 49	1,289 ± 186	11	0.001
Control	26	27.3 ± 2.1	208 ± 35	1,189 ± 367		
90° C. Treated	29	27.1 ± 2.1	197 ± 27	1,018 ± 276	22	0.001
Control	21	28.0 ± 3.5	191 ± 30	792 ± 437		
95° C. Treated	12	28.1 ± 2.6	205 ± 51	386 ± 175	11	0.001
Control	14	27.6 ± 1.6	207 ± 38	309 ± 161		

Fig. 1 shows more vividly how these results compare. The survival time of our control groups was found significantly altered; depending on the temperature used, p was less than 0.001.

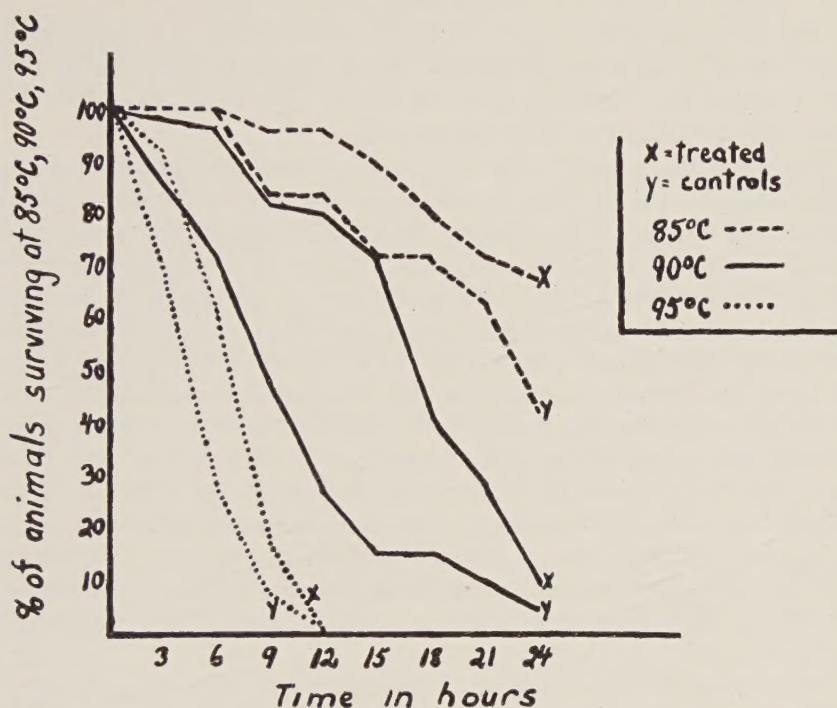


Fig. 1.—Comparing survival time of treated and control animals.

DISCUSSION

The mean survival time of our controls is significantly influenced by relatively minor temperature differences. Six hours after burning all the animals of the 85° C. group were alive as compared to only 28 per cent of our 95° C. group. This could be due to a gradual increase in fluid loss with increasing burn temperature. Although we did not measure for fluid loss in our experiments, we could see no obvious difference in swelling of the excised burned areas at the various temperatures. Indeed it is well known that edema formation decreases with increasing temperature and duration of burn, because of local circulatory arrest. Prinzmetal¹⁷ showed that rats the legs of which were dipped in a water bath of 100° C. for 2 to 3 minutes, exuded 2 per cent of their body weight, while other rats burned at 75° C. for 10 seconds lost 5 per cent of their body weight. Edema was far more profound in the latter case. Nevertheless, there was a shorter survival time in the more severely burned group. It may, therefore be concluded that toxic and/or nervous influences could be important factors in causing decreased survival time.

The investigations of van Canegham,³ Simonart,²⁰ and Allgöwer and Mégevand² point to the fact that proteins heated to above 80° C. may induce toxic reactions. These last investigators² injected heated blood coagula intraperitoneally in

rabbits. Thirty animals received 50 to 100 c.c. of blood heated to 100° for one hour. Almost 50 per cent of these died within 48 hours without excessive peritoneal exudation. Intraperitoneal injection of blood heated for one hour at 80° C. caused no death in 11 rabbits during the same period.

The significant difference in survival time between our rats burned at 95° C. and 85° C. thus is apparently not due to increased fluid loss at the former burn temperature. This too seems to suggest that some toxic and/or nervous factors play a part at the higher temperature.

By using the composite survival times of the first 24 hours we established a significant life prolongation time in those rats (150 to 250 Gm.) intravenously treated with 0.2 mg. Isoindoline. Ross and Herczeg¹⁸ in a small series of animals noted that a critical dose range of ganglion blockers including Isoindoline was required to produce a certain degree of protection in pretreated rats submitted to drum trauma. One to 5.0 mg. per kilogram of Isoindoline was found to be the optimum dosage, with higher and lower dose levels causing a decrease in the protective action, but this dose was injected intraperitoneally. Although the danger of puncturing the intestine or viscera is slight, there is irregularity in absorption, particularly if the circulation is impaired, as may be the case in trauma. Also, the virtue of using the 24-hour period as in the case of these authors has never been shown to possess particularly advantage or validity. We can see in Table I that, in the 90° C. group, 80 per cent of the controls were dead 12 hours post burn as compared to 26 per cent of the treated animals. Thus if none had survival 24 hours it would be impossible to detect any subtle differences.

The prolongation of mean survival time by ganglion-blocking agents seems to suggest their attenuating effect on nervous shock. Blocking of autonomic ganglia decreases the sympathetic and parasympathetic discharge. Isoindoline has been shown empirically to have a greater blocking action on the sympathetic than the parasympathetic nervous systems.^{11, 13, 15, 19} It has to be kept in mind, however, that blocking agents also cause hemodynamic changes, thus reducing exudation, that is, fluid loss. The data of Phemister and Laestar¹⁴ and Wang and Overman²¹ lend support to the view that both excessive vasoconstrictor tone due to an increased discharge of nociceptive stimuli from the burned area and fluid loss into the affected area are moderated by the hypotensive drug.

Using Isoindoline, we were able to confirm Laver's findings that treatment with a ganglion-blocking agent post burn had a significant effect on survival time. In most other shock procedures, pretreatment is the only effective method. Administration of the drug immediately after burning may be somewhat equated to pretreatment in other shock forms, since the burn gradually initiates the process (fluid loss, nervous excitation, and possibly toxin resorption), which in the other forms of shock takes place almost at once.

The findings that treatment after burn trauma is effective could have some practical value, since the application of a ganglion-blocking agent may delay the critical survival time, which is in great part ultimately determined by the rate of fluid loss.

SUMMARY

1. Increasing the temperature of a hot water burn in rats from 85° C. to 95° C. resulted in a significant decrease of mean survival time.
2. Treatment with Isoindoline (0.2 mg. per rat) immediately after burning resulted in a significant increase of mean survival time in all temperature ranges tested, the effect of the blocking agent being most obvious at 90° C.

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CURRICULUM VITAE

Ich, Eugene Schwalb, Burger der Vereinigten Staten von Amerika, wurde am 7. August 1938 in Prague, Czechoslovakia, geboren.

In April 1941 übersiedelte ich nach Amerika, wo ich die Primar Schule in New York eintrat. In 1947 begann ich das Gymnasium (De Witt Clinton High School) wo ich in 1950 die Matura mit Erfolg bestand. In Fruhling 1950 setzte ich meine Studien am City College of New York fort und schloss sie 1953 erfolgreich mit dem "Bachelor of Science" Diplom ab.

Anschliessand begab ich mich in die Schweiz um an der Universitat von Basel Medizin zu studieren. In Fruhling 1954 bestand ich mit Erfolg das zweite propadeutische Examen und promovierte am 5. December 1958.